Background & objectives: Overproduction of lipid peroxidation byproducts and disturbances in antioxidant defense system have been implicated in the pathogenesis of several diseases including oral cancer. Though several studies have been done on the level of lipid peroxidation and antioxidants in oral cancer patients, there are no reports in patients with various clinical stages of oral squamous cell carcinoma. We carried out this study to assess the level of oxidative stress in oral cancer patients with various clinical stages.

Methods: Blood samples of 48 adult male oral cancer patients with various clinical stages of oral cancer (stage II to stage IV, 16 of each) and 16 age and sex matched healthy subjects were collected. Plasma and erythrocytes levels of thiobarbituric acid reactive substances (TBARS), vitamin E, reduced glutathione (GSH), and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assayed using specific colorimetric methods. The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Student’s t-test.

Results: Elevated lipid peroxidation and decline in non-enzymatic and enzymatic antioxidants status were noticed in oral cancer patients as compared to healthy subjects. The TBARS levels were gradually increased whereas antioxidants were gradually reduced from stage II to stage IV of oral cancer patients.

Interpretation & conclusion: The altered lipid peroxidation in plasma and erythrocytes of oral cancer patients may be related to their compensatory changes in the antioxidants defense system.

Key words Antioxidants - lipid peroxidation - oral cancer

Oral cancer is a major form of cancer worldwide and is one of the most common malignancy in India accounting for 30-40 per cent of all cancers¹,². Squamous cell carcinoma of the oral cavity is responsible for considerable morbidity and mortality in India where 60,000 new cases of oral cancer are reported to occur every year³. Tobacco chewing with betel quid, tobacco smoking and alcohol consumption...
are the most important aetiological factors associated with the incidence of oral cancer in India.

Reactive oxygen species plays an effective role in the pathogenesis of different pathological diseases including cancer. Free radical induced lipid peroxidation causes a loss of cell homeostasis by modifying the structure and functions of cell membrane. The most important characteristic of lipid peroxidation is to cause a considerable DNA-MDA adducts by interacting with cellular DNA. However, mammalian cells possess elaborate antioxidant defense mechanisms to neutralize the deleterious effects of free radical induced lipid peroxidation.

Enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and non-enzymatic antioxidants [vitamin E, reduced glutathione (GSH)] act synergistically with one another to detoxify the effects of lipid peroxidation. We have earlier demonstrated the status of lipid peroxidation and antioxidant defense mechanism in plasma and erythrocytes of oral cancer patients. Subapriya et al. have reported enhanced lipid peroxidation with decline in antioxidants in venous blood of patients with oral squamous cell carcinoma at different intraoral sites. However, there are no reports on lipid peroxidation and antioxidant status in patients with various clinical stages of oral squamous cell carcinoma. The present study was therefore, undertaken to evaluate the status of lipid peroxidation and antioxidants in plasma and erythrocytes of oral cancer patients with various clinical stages.

**Material & Methods**

Blood samples were obtained consecutively from 48 male oral cancer patients admitted during 2001-2003, in Raja Muthiah Dental College and Hospital (RMDC&H), Annamalai University, Annamalai Nagar, Tamil Nadu, India. The patients were categorized into three different groups of 16 each (stages II, III and IV) according to TNM (tumour, node, metastasis) system of cancer classification. Sixteen age matched healthy males were also investigated as control. Appropriate control subjects were selected in and around Annamalai Nagar. The subjects who were diagnosed as cancer-free were categorised as control subjects. The controls were healthy subjects not habituated to tobacco chewing and smoking and were of the same age, sex and socio-economic strata as the oral cancer patients. The subjects were ranging in age from 40-60 yr (control: 51.3 ± 4.6; oral cancer patients: 53.2 ± 5.1). As only 16 patients with stage IV carcinoma could be admitted and included during the study period the number in other subgroups with stages II and III and control group was also restricted to 16 in each group. So the first 16 patients with stage II or III were included. Study protocol was approved by the ethics committee of RMDC & H.

Oral tumour tissues obtained at the time of surgery from buccal mucosa of patients were immediately fixed in 10 per cent formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The tumours were histopathologically confirmed as well, moderately and poorly differentiated squamous cell carcinoma.

Blood samples (6 ml) were obtained from patients and controls by venous arm puncture and the plasma was separated by centrifugation at 1000 g for 15 min. After plasma separation, the buffy coat was removed and the packed cells were washed three times with physiological saline. A known volume of erythrocytes was lysed with hypotonic phosphate buffer, pH 7.4. The haemolysate was separated by centrifugation at 3500 g for 15 min at 20°C. The erythrocyte membrane was prepared by the method of Dodge et al. with a change in buffer according to Quist. TBARS and antioxidants estimations were carried out in plasma, erythrocytes and erythrocyte membranes of healthy subjects and oral cancer patients.

Thiobarbituric acid reactive substances (TBARS) released from the endogenous lipoperoxides, reflecting the lipid peroxidation processes, were assayed in plasma, and in erythrocytes and erythrocyte membranes. Vitamin E level was determined by the method of Desai.
The reduced glutathione was determined by the method of Beutler and Kelly\textsuperscript{21}. The technique involves protein precipitation by meta-phosphoric acid and spectrophotometric assay at 412 nm of the yellow derivative obtained by the reaction of the supernatant with 5-5’-dithiobis-2-nitrobenzoic acid. The erythrocyte membrane protein was measured by the method of Lowry et al\textsuperscript{22}.

Cytoplasmic Cu/Zn superoxide dismutase activity was assayed by the method of Kakkar et al\textsuperscript{23}. One unit of enzyme is taken as the amount of enzyme required to give 50 per cent inhibition of nitro blue tetrazolium (NBT) reduction. The activity of catalase was assayed by the method of Sinha\textsuperscript{24}, based on the utilization of H\textsubscript{2}O\textsubscript{2} by the enzyme. The colour developed was read at 620 nm. One unit of the enzyme is expressed as µ moles of H\textsubscript{2}O\textsubscript{2} utilized per minute. Glutathione peroxidase activity was assayed by the method of Rotruck et al\textsuperscript{25} based on the utilization of reduced glutathione by the enzyme. One unit of enzyme is expressed as µ moles of GSH utilized per minute.

Statistical analysis: The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Student’s t-test.

Results & Discussion

A total of 48 patients and 16 control subjects were selected for the study. The patients were categorized into three groups of 16 each, based on their clinical stages of tumor (Stages II, III, and IV), according to TNM system of cancer classification. The habit of tobacco chewing, smoking and alcohol consumption were common in oral cancer patients. The clinical status of patients as confirmed by histopathological examination was found to be well-differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma. Healthy subjects were not habituated to tobacco chewing, smoking and alcoholic consumption, and were free from any systemic diseases. Both normal subjects and oral cancer patients were males ranging in age from 40-60 yr (control: 51.3 ± 4.6; oral cancer patients: 53.2 ± 5.1).

The level of TBARS was significantly increased in plasma, erythrocytes and erythrocyte membranes of oral cancer patients as compared to healthy subjects, and were gradually increased from stage II to stage IV of oral cancer patients (Table I). The levels of vitamin E and reduced glutathione were significantly decreased in oral cancer patients as compared to healthy subjects, and were gradually decreased from stage II to stage IV of oral cancer patients (Table II). The activities of superoxide dismutase, catalase and glutathione peroxidase were also significantly decreased in oral cancer patients as compared to healthy subjects, and were gradually decreased from stage II to stage IV of oral cancer patients (Table III).

Byproducts of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes\textsuperscript{26}. Lipid peroxidation byproducts formed under physiological and pathological

<table>
<thead>
<tr>
<th>Table I. TBARS levels in plasma, erythrocytes, and erythrocyte membranes of healthy subjects and oral cancer patients with various clinical stages</th>
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<tr>
<td>Parameters</td>
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<tr>
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<tr>
<td>Plasma TBARS (n mol/ml)</td>
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<tr>
<td>Erythrocyte TBARS (p mol/mg Hb)</td>
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<tr>
<td>Erythrocyte membrane TBARS (n mol/mg protein)</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=16)
* P<0.01, **<0.001 compared to healthy subjects
* P<0.01, **<0.001 compared to stage II
* P<0.001 compared to stage III
conditions are scavenged by non enzymatic and enzymatic antioxidants. An imbalance between antioxidant defense mechanism and lipid peroxidation processes results in cell and tissue damage. Lower levels of plasma and erythrocyte membrane vitamin E have been reported in various pathological conditions including oral cancer. A positive correlation between vitamin E and lipid peroxidation has been reported in oral cancer patients and patients with oral squamous cell carcinoma at different intraoral sites. Kolanjiappan et al have reported an increase in vitamin E and glutathione levels in tumour tissues of patients with various clinical stages of oral squamous cell carcinoma. Buzby et al suggested that host tumour cells sequester essential nutrients from the circulation to meet the demand of growing tumour. Thus, the observed decrease in vitamin E and reduced glutathione in plasma and erythrocytes of oral cancer patients can be due to utilization of these antioxidants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy subjects</th>
<th>Oral cancer patients</th>
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<tbody>
<tr>
<td>Plasm vitamin E (mg/dl)</td>
<td>1.20 ± 0.11</td>
<td>1.08 ± 0.10</td>
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<tr>
<td>Plasma reduced glutathione (mg/dl)</td>
<td>50.64 ± 5.17</td>
<td>44.09 ± 5.62</td>
</tr>
<tr>
<td>Erythrocyte membrane vitamin E (µg/mg protein)</td>
<td>1.84 ± 0.17</td>
<td>1.65 ± 0.13</td>
</tr>
<tr>
<td>Erythrocyte reduced glutathione (mg/dl)</td>
<td>50.15 ± 4.12</td>
<td>45.24 ± 3.86</td>
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</table>

Values are mean ± SD (n=16)

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<tr>
<th>Parameters</th>
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<th>Oral cancer patients</th>
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</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (U/l)</td>
<td>223.84 ± 17.70</td>
<td>204.10 ± 13.73</td>
</tr>
<tr>
<td>Superoxide dismutase (U/ml)</td>
<td>4.19 ± 0.31</td>
<td>3.61 ± 0.72</td>
</tr>
<tr>
<td>Catalase (U/l)</td>
<td>0.54 ± 0.03</td>
<td>0.51 ± 0.03</td>
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<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy subjects</th>
<th>Oral cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (U/g Hb)</td>
<td>22.32 ± 1.86</td>
<td>20.04 ± 2.10</td>
</tr>
<tr>
<td>Superoxide dismutase (U/mg Hb)</td>
<td>2.29 ± 0.17</td>
<td>2.08 ± 0.08</td>
</tr>
<tr>
<td>Catalase (U/mg Hb)</td>
<td>1.76 ± 0.12</td>
<td>1.59 ± 0.16</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=16)

- µ moles of GSH utilized/min
- Amount of enzyme required to inhibit 50 per cent nitroblue tetrazolium reduction
- µ moles of H₂O₂ utilized/min
- µ moles of H₂O₂ utilized/sec

P<0.01, **<0.001 compared to healthy subjects
P<0.01, **<0.001 compared to stage II
P<0.001 compared to stage III
by tumour tissues or to compromise the excessive oxidative stress in circulation.

The antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase serve as the backbone of cellular antioxidant defense mechanisms. Lowered activities of these enzymes have been reported in various pathological conditions including oral carcinogenesis. Our results support these observations.

In the present study the levels of circulating TBARS and antioxidants were found to be associated with the tumour stages of patients. Measurement of lipid peroxidation and antioxidants in circulation of oral cancer patients may thus be helpful in assessing the tumour stages of oral squamous cell carcinoma.

Acknowledgment

Authors thank Dr C.R. Ramachandran, Dean, Faculty of Dentistry, Raja Muthiah Dental College and Hospital (RMDC&H), Annamalai University for his help in categorizing oral cancer patients and carrying out histopathological studies.

References


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